

# Transfection, pool maintenance, and drug addition

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An abbreviated version of this protocol was published in eLIFE in Jul 2018

Pooled genome-wide CRISPR screening for basal and context-specific fitness gene essentiality in *Drosophila* cells

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## Detailed protocol

Link to detailed protocol: <https://currentprotocols.onlinelibrary.wiley.com/doi/abs/10.1002/cpmb.111>

## Pooled CRISPR Screens in *Drosophila* Cells

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### Abstract

High-throughput screens in *Drosophila melanogaster* cell lines have led to discovery of conserved gene functions related to signal transduction, host-pathogen interactions, ion transport, and more. CRISPR/Cas9 technology has opened the door to new types of large-scale cell-based screens. Whereas array-format screens require liquid handling automation and assay miniaturization, pooled-format screens, in which reagents are introduced at random and in bulk, can be done in a standard lab setting. We provide a detailed protocol for conducting and evaluating genome-wide CRISPR single guide RNA (sgRNA) pooled screens in *Drosophila* S2R+ cultured cells. Specifically, we provide step-by-step instructions for library design and production, optimization of cytotoxin-based selection assays, genome-scale screening, and data analysis. This type of project takes ~3 months to complete. Results can be used in follow-up studies performed in vivo in *Drosophila*, mammalian cells, and/or other systems. © 2019 by John Wiley & Sons, Inc.

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1. Viswanatha, R. and Perrimon, N. (2020). Transfection, pool maintenance, and drug addition. Bio-protocol Preprint. [bio-protocol.org/prep685](https://bio-protocol.org/prep685).
2. Viswanatha, R., Li, Z., Hu, Y. and Perrimon, N. (2018). Pooled genome-wide CRISPR screening for basal and context-specific fitness gene essentiality in *Drosophila* cells. eLIFE. DOI: [10.7554/eLife.36333](https://doi.org/10.7554/eLife.36333)

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